on the presence of functional mast cells. These results are similar to those reported for other systems.

For example, in ovalbumin (OVA)–sensitized, iteratively challenged mice, mast cells had no effect on eosinophil influx in bronchoalveolar lavage fluid.[4] Like their congenic littermates, after sensitization and airway challenge with OVA, mast cell–deficient mice had allergic antibody and pulmonary eosinophilic responses.[3] On the other hand, in sensitized mast cell–deficient mice, OVA challenge produced fewer eosinophils in the bronchoalveolar lavage fluid and lungs than in similarly sensitized and challenged congenic littermates.[2] However, others[3,5] contended that in the murine asthmatic pulmonary eosinophilic response, mast cells might be of greatest importance when relatively weak stimulants or inducers are used to induce the response. They pointed out that because Kung et al[2] used attenuated sensitization and challenge protocols, the number of eosinophils might have been significantly lower in their study than in others.

We also used attenuated sensitization and challenge protocols to examine eosinophilic conjunctival inflammation. Sensitization was with 1 intracutaneous injection of RW adsorbed on alum on day 0, followed by 1 intraperitoneal injection on day 7; challenge was with only 1 exposure to RW in PBS on day 18. Although these attenuated protocols did not induce detectable anti-RW IgE in serum, we found that mast cell–deficient mice were capable of having eosinophilic conjunctival inflammation similar to that seen in their congenic littermates.

Human conjunctival epithelial cells expressed eotaxin *in vivo*,[6,7] and conjunctival fibroblasts produced eotaxin *in vitro*,[8] suggesting that conjunctival epithelial cells, fibroblasts, or both might be implicated in the eosinophilic conjunctival inflammation seen in allergic conjunctivitis. Investigations are underway in our laboratory to identify the cells implicated in the eosinophilic conjunctival inflammation of allergic conjunctivitis.

In summary, we showed that mast cell–deficient mice exposed to sensitization and eyedrop challenge had eosinophilic conjunctival inflammation similar to that seen in their congenic littermates. Although this does not exclude mast cell contributions to other aspects of late-phase allergic conjunctivitis, our finding indicates that these cells do not play an essential role in the development of eosinophilic conjunctival inflammation in mice sensitized and challenged as described in this report.

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Irritant skin test reactions to common vaccines

To the Editor:

Concerns about possible allergic reactions to immunizations are frequently raised by both patients/parents and primary caretakers. Estimates of true allergic reactions to routine vaccines range from 1 per 50,000 doses for diphtheria-tetanus-pertussis vaccine to about 1 per 500,000 to 1,000,000 doses for most other vaccines.[1] Although these per-dose estimates suggest that reactions are extraordinarily rare, the large number of doses administered makes this a relatively common clinical problem.

Skin testing for immediate-type hypersensitivity is indicated for some patients with suspected allergic reactions to immunizations.[2,3] Standard skin testing to vaccines involves the use of both prick (puncture) and intradermal (intracutaneous) skin tests at varying concentrations. However, the interpretation of these skin test results is complicated in many instances by irritant reactions. Although it is clear from clinical experience that irritant reactions do occur to some vaccines, the frequency with which they occur has never been formally evaluated.

Twenty healthy adult volunteers with no history of food or drug allergy or adverse vaccine reactions were tested to 10 common vaccines (Table I). Each vaccine was tested at a full-strength skin prick dose with a bifurcated needle and at 1:100, 1:10, and full-strength intradermal doses. All test results were read at 15 minutes and compared with results with positive (histamine) and negative (diluent) controls.

All antihistamines were withheld for 7 days before skin testing. For intradermal tests, 0.02 mL was injected by using a 0.5-mL syringe with a 27-gauge needle. Dilutions were prepared at the time of testing with normal saline with 0.03% human serum albumin.

After skin testing, subjects were observed for 1 hour. They were instructed to call with any possible late reactions so that a clinic visit could be arranged. Follow-up telephone calls were conducted at 48 to 72 hours and 7 days after skin testing to assess for any delayed reactions. Where possible, those with delayed reactions were seen by the investigator. The protocol was approved by the Institutional Review Board of the Johns Hopkins Bloomberg School of Public Health, and all subjects signed informed consent forms.

Any skin test result with a wheal that exceeded that produced by the negative control by more than 3 mm was considered positive. The frequency of such reactions to each vaccine at each concentration was recorded. A nonirritating concentration for each vaccine was established by identifying the highest concentration at which all participants had a wheal response of less than 3 mm greater than that elicited by the negative control.

Twenty-three subjects participated in the study, 15 female and 8 male patients between the ages of 20 and 59 years. Sixteen underwent testing to all 10 vaccines, and 7 underwent testing to a subset of vaccines, primarily because no influenza vaccine was available at study initiation. Each vaccine was tested at all concentrations, even if a positive result occurred at a lesser concentration.

No positive (irritant) result occurred with skin prick testing to any vaccine. At the 1:100 concentration, all intradermal test results were negative except for 1 subject with weakly positive test results to both diphtheria–tetanus–acellular pertussis (DTaP) and diphtheria-tetanus (DT) vaccines and 3 with positive test results to influenza vaccine (Table II). At the 1:10 concentration, positive test results occurred in 1 (5%) subject to DT vaccine, in 1 (5%) subject to Prevnar (Wyeth, Collegeville, Pa), in 2 (10%) subjects to DTaP vaccine, and in 11 (55%) subjects to influenza vaccine. At full strength, positive intradermal test results occurred with all vaccines except hepatitis A and inactivated poliomyelitis vaccine and were most common with measles, mumps, and rubella (MMR; 20/20 [100%]); varicella (20/20 [100%]); influenza (13/20 [65%]); and *Haemophilus influenzae* b (Hib; 5/20 [25%]) vaccines. All subjects with a positive result at one concentration also had positive results at all higher concentrations. No subject had persistence of their immediate-type reactions, with all positive test results resolving over 1 to 24 hours. On follow-up assessment, 1 subject was unable to be contacted. All other subjects were contacted by telephone, and 12 subjects were seen by the investigator to assess delayed-type reactions. At the 1:100 concentration, there were 2 delayed reactions to influenza vaccine and 1 to DTaP vaccine at 48 to 72 hours. Delayed reactions were common for most vaccines at the 1:10 and full-strength concentrations at the 48- to 72-hour follow-up, many of which were still present at the 7-day follow-up (Table II). For those that were directly observed, reactions ranged from mild erythema to 18 mm of induration. The largest and most frequent delayed reactions occurred to DTaP, DT, and influenza vaccine, followed by Prevnar, varicella, and Hib vaccines.

TABLE I. Specific vaccines tested

Vaccine	Trade name	Manufacturer
DtaP	Infanrix	GlaxoSmithKline, Glenval, Belgium
DT	Diphtheria and Tetanus Toxoids Adsorbed USP (For Pediatric Use)	Sanofi Pasteur, Swiftwater, Pa
MMR	M-M-R II	Merck & Co, Inc, Whitehouse Station, NJ
Hib	ActHIB	Sanofi Pasteur, Lyon, France
Hepatitis B	Engerix-B	GlaxoSmithKline, Glenval, Belgium
IPV	IPOL	Sanofi Pasteur, Lyon, France
Varicella	Varivax	Merck & Co, Inc, Whitehouse Station, NJ
Hepatitis A	Havrix	GlaxoSmithKline, Glenval, Belgium
Pneumococcal	Prevnar	Wyeth, Collegeville, Pa
Influenza virus	Fluzone	Sanofi Pasteur, Swiftwater, Pa

DTaP, Diphtheria–tetanus–acellular pertussis; *DT*, diphtheria-tetanus; *MMR*, measles, mumps, and rubella; *Hib*, *Haemophilus influenzae* b; *IPV*, inactivated poliomyelitis vaccine.

All vaccines have the potential to cause allergic reactions, and several vaccine components could be allergenic, including the vaccine itself, preservatives, and stabilizers.[4] Specific vaccine components that have been implicated in vaccine reactions include egg protein, gelatin, neomycin, thimerosal, and other additives.[2-8] Specific testing, especially to egg and gelatin, might be very helpful in the evaluation of some vaccine reactions,[6,7] whereas in other cases specific testing of vaccine components might be unavailable or unrevealing. In those instances skin testing with the intact vaccine could be the only diagnostic option.[2,3,8]

Although guidelines for the evaluation of patients with suspected allergic reactions to vaccines have been proposed,[2,3] there are no standardized approaches. These guidelines typically recommend skin prick testing with the full-strength vaccine and intradermal testing with a 1:100 dilution. However, in clinical practice it is not uncommon for allergists to use intradermal skin tests at higher concentrations, up to and including full-strength undiluted vaccine.

We reported in 1992 that 3 of 6 adult control subjects had irritant effects to a 1:100 concentration of MMR, at which point we took all intradermal testing out of our MMR testing protocol.[9] Aside from that limited sample, knowledge regarding the irritant effects of different vaccines is based solely on clinical experience. We therefore undertook this study in an effort to determine the frequency with which irritant reactions occur to 10 common vaccines.

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TABLE II. Immediate- and delayed-type responses to intradermal testing

	Immediate reactions (n = 20)			Delayed reactions (n = 19)	
	No. positive (n = 20)	Wheal (range; mm)	Erythema (range; mm)	48-72 h (no. positive)	7 d (no. positive)
DT					
1:100	1	3	3	0	0
1:10	1	5	15	7	2
Full strength	2	4-9	15-28	11	7
DTaP					
1:100	1	5-16	3	1	0
1:10	2	3-4	6-18	8	5
Full strength	4	5-16	15-20	12	9
Hepatitis A					
1:100	0	—	—	0	0
1:10	0	—	—	1	0
Full strength	0	—	—	2	1
Hepatitis B					
1:100	0	—	—	0	0
1:10	0	—	—	1	0
Full strength	1	3	6	2	1
Hib					
1:100	0	—	—	0	0
1:10	0	—	—	5	3
Full strength	5	3-5	3-15	8	6
Influenza					
1:100	3	3-5	4-8	2	0
1:10	11	4-6	15-20	10	2
Full strength	13	4-8	14-38	10	3
IPV					
1:100	0	—	—	0	0
1:10	0	—	—	1	0
Full strength	0	—	—	1	0
MMR					
1:100	0	—	—	0	0
1:10	0	—	—	1	1
Full strength	20	4-9	7-30	5	2
Prevnar					
1:100	0	—	—	0	0
1:10	1	4	8	6	2
Full strength	3	3-4	5-20	8	4
Varicella					
1:100	0	—	—	0	0
1:10	0	—	—	6	2
Full strength	20	4-8	7-30	8	3

No immediate or delayed responses occurred to skin prick testing.
DT, Diphtheria-tetanus; *DTaP*, diphtheria–tetanus–acellular pertussis; *Hib*, *Haemophilus influenzae* b; *IPV*, inactivated poliomyelitis vaccine; *MMR*, measles, mumps, and rubella.

Some very clear patterns emerge from the results of this study. First, skin prick testing, even with full-strength vaccine, is unlikely to be complicated by irritant effects. Likewise, irritant effects are very uncommon with intradermal testing at 1:100 dilutions. Even though this did occur with 1 subject to DT and DTaP vaccines, these were small positive test results. It is also clear that irritant reactions are rare at all concentrations for some vaccines, including hepatitis A, hepatitis B, and inactivated poliomyelitis vaccines. With influenza, 3 of 20 subjects had positive results at the 1:100 concentration, with the largest being a 5-mm wheal with an 8-mm flare. Although not common, the clinician should be aware that these reactions might occur, even at this concentration, especially given the fact that this is a concentration that has been formally recommended for testing patients with egg allergy before administration of an influenza vaccine.[10]

At the 1:10 and full-strength concentrations, irritant reactions were very common for some vaccines. Most problematic were full-strength MMR and varicella vaccines, both full-strength and 1:10 influenza vaccines, and, to a lesser extent, full-strength DTaP, DT, Hib, and Prevnar vaccines. Whether these higher concentrations would ever be needed from a clinical standpoint is essentially unknown, aside from the data published on MMR and influenza,[9,11] which showed that these vaccines

could be safely administered after a negative full-strength skin prick test result for MMR vaccine and a negative 1:100 intradermal test result for influenza vaccine.

The delayed-type reactions that were seen will not affect the interpretation of skin test results but should be recognized as potential complications of vaccine skin testing. These were uncommon with testing at the 1:100 dilutions but were very common for some vaccines at the 1:10 and full-strength concentrations.

It is important to recognize several limitations to this study. First, the numbers of subjects tested per vaccine are relatively small. Although the results provide a representation of the relative likelihood of a vaccine having irritant effects, they cannot be taken as conclusive.

Second, these vaccines are mostly used in children, but these data were collected only in adults because of safety concerns and ethical issues. It is possible that children, especially those with atopy, would be more reactive than the adults included in this protocol and that the frequency of irritant effects might be higher than those reported here.

Third, it is possible that some of these irritant reactions did truly represent positive immediate-type allergic responses, although this is unlikely given the population studied and the rarity of allergic reactions to vaccines.

Finally, for many of these vaccines, multiple brands are available from different manufacturers. Although irritant effects are likely to be similar for the different formulations of the same vaccine, this might not always be the case.

Even with these limitations, these results provide clinicians with useful guidelines as to choosing appropriate concentrations for vaccine skin testing. Clearly, intradermal skin testing with full-strength MMR vaccine or Varivax (Merck & Co, Inc, Whitehouse Station, NJ) will routinely be complicated by irritant responses, and intradermal testing with full-strength or 1:10 influenza would be of limited value. Aside from the need to cautiously interpret testing with influenza at the 1:100 concentration, for all other vaccines, this concentration would be expected to produce few, if any, irritant responses.

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